

CLAIMS

1. A process for producing recombinant cytokine comprising producing a gene recombinant silkworm that incorporates cytokine gene in its chromosomes, producing
5 recombinant cytokine protein in the silk glands or cocoon and silk thread of the resulting gene recombinant silkworm, and recovering the cytokine from the silk glands or cocoon and silk thread.
2. A process for producing recombinant cytokine
10 according to claim 1 wherein a cytokine gene coupled downstream from a promoter specifically expressed in silk glands is incorporated in a chromosome.
3. A process for producing recombinant cytokine according to claim 2 wherein the promoter specifically
15 expressed in silk glands is a sericin gene promoter.
4. A process for producing recombinant cytokine according to claim 2 wherein the promoter specifically expressed in silk glands is a fibroin H chain gene promoter.
- 20 5. A process for producing recombinant cytokine according to any of claims 1 through 4 wherein cytokine gene is incorporated in silkworm chromosomes using DNA originating in a transposon.
- 25 6. A process for producing recombinant cytokine according to claim 5 wherein the cytokine gene is located between a pair of inverted terminal sequences originating in a transposon.
- 30 7. A process for producing recombinant cytokine according to claim 5 or 6 wherein the DNA originating in a transposon originates in an insect.
8. A process for producing recombinant cytokine according to claim 7 wherein the transposon originates in piggyBac transposon originating in a lepidopteron.
- 35 9. A process for producing recombinant cytokine according to any of claims 1 through 8 wherein the cytokine gene is interferon gene or colony stimulating factor gene.

10. A process for producing recombinant cytokine according to claim 9 wherein the interferon gene or colony stimulating factor gene is feline interferon- ω gene, human interferon- β gene or feline granulocyte colony stimulating factor gene.

11. A process for producing recombinant cytokine according to any of claims 1 through 3 wherein cytokine is extracted from cocoon and silk thread by using an aqueous solvent.

12. A gene recombinant silkworm in which a cytokine gene has been inserted into a chromosome and cytokine is produced in silk glands or cocoon and silk thread.

13. A gene recombinant silkworm according to claim 12 wherein the cytokine gene inserted into a chromosome is an interferon gene or colony stimulating factor gene.

14. A gene recombinant silkworm according to claim 13 wherein the interferon gene or colony stimulating factor gene inserted into a chromosome is feline interferon- ω gene, human interferon- β gene or feline granulocyte colony stimulating factor gene.

15. A vector for inserting an exogenous gene into silkworm chromosomes in which a cytokine gene is coupled downstream from a promoter that is specifically expressed in silk glands.

16. A vector for inserting an exogenous gene into silkworm chromosomes according to claim 15 wherein the promoter is sericin gene promoter.

17. A vector for inserting an exogenous gene into silkworm chromosomes according to claim 15 wherein the promoter is a fibroin H chain gene promoter.

18. A vector for inserting an exogenous gene into silkworm chromosomes according to any of claims 15 through 17 wherein the cytokine gene is located between a pair of inverted terminal sequences originating in a transposon.

19. A vector for inserting an exogenous gene into

silkworm chromosomes according to any of claims 15 through 18 wherein the cytokine gene is an interferon gene or a colony stimulating factor gene.

5 20. A vector for inserting an exogenous gene into silkworm chromosomes according to claim 19 wherein the interferon gene or colony stimulating factor gene is feline interferon- ω gene, human interferon- β gene or feline granulocyte colony stimulating factor gene.

10 21. A gene cassette for expressing an exogenous protein comprising (1) a promoter expressed in silk glands, and (2) a gene coupled downstream from (1) in which the 5' terminal portion of fibroin H chain gene is fused to the 5' side of an exogenous protein structural gene.

15 22. A gene cassette for expressing an exogenous protein comprising (1) a promoter expressed in silk glands, and (2) a gene coupled downstream from (1) in which the 3' terminal portion of fibroin H chain gene is fused to the 3' side of an exogenous protein structural
20 gene not containing a stop codon, or a gene cassette for expressing an exogenous protein comprising (1) a promoter expressed in silk glands, and (2) a gene coupled downstream from (1) in which an exogenous protein structural gene is fused to the 3' side of the 3'
25 terminal portion of fibroin H chain gene.

23. A gene cassette for expressing an exogenous protein comprising (1) a promoter expressed in silk glands, and (2) a gene coupled downstream from (1) in which the 5' terminal portion of fibroin H chain gene is
30 fused to the 5' side of an exogenous protein structural gene not containing a stop codon, and in which the 3' terminal portion of fibrin H chain gene is fused to the 3' side of the structural gene.

35 24. A gene cassette according to claim 21 or 23 wherein the 5' terminal portion of the fibroin H chain gene contains a first exon, first intron and a portion of a second exon of fibroin H chain gene.

25. A gene cassette according to claim 24 wherein the portion where the first exon and second exon of the fibroin H chain gene are joined is a secretion signal gene region of fibroin H chain gene.

5 26. A gene cassette according to claim 25 wherein the promoter expressed in silk glands of (1) and the 5' terminal portion of fibroin H chain gene coupled downstream from (1) are the DNA shown in SEQ. ID No. 22 and SEQ. ID No. 23.

10 27. A gene cassette according to claim 22 or 23 wherein the 3' terminal portion of the fibroin H chain gene contains at least one codon that encodes cysteine.

15 28. A gene cassette according to claim 27 wherein the 3' terminal portion of the fibroin H chain gene is the DNA shown in SEQ. ID No. 24.

20 29. A gene cassette according to any of claims 21 through 28 wherein the promoter expressed in silk glands is at least one promoter selected from fibroin H chain gene promoter, fibroin L chain gene promoter and sericin gene promoter.

25 30. A gene cassette according to any of claims 21 through 29 wherein at least one poly A addition region selected from a poly A addition region of fibroin H chain gene, a poly A addition region of fibroin L chain gene and a poly A addition region of sericin gene is present downstream from a gene cassette for expressing an exogenous protein according to any of claims 21 through 29.

30 31. A gene cassette for inserting a gene into chromosomes of insect cells comprising inverted repetitive sequences of a pair of piggyBac transposons present on both sides of a gene cassette for expressing an exogenous protein according to any of claims 21 through 30.

35 32. An expression vector for insect cells that contains a gene cassette for expressing an exogenous protein according to any of claims 21 through 31.

33. A gene insertion vector for insect cells that contains a gene cassette for inserting a gene into chromosomes of insect cells according to claim 31.

5 34. A process for producing an exogenous protein comprising inserting a vector for insect cells according to claim 32 or 33 into insect cells.

35. A process for producing an exogenous protein according to claim 34 wherein the insect cells originate in a lepidopteron.

10 36. A process for producing an exogenous protein according to claim 35 wherein the insect cells originate in silkworm moths (*Bombyx mori*).

15 37. A process for producing an exogenous protein according to claim 36 wherein the insect cells are silk gland cells of silkworm moths (*Bombyx mori*).

20 38. A process for producing an exogenous protein comprising producing a recombinant silkworm in which a gene cassette for expressing an exogenous protein according to any of claims 21 through 31 is inserted into a chromosome using a gene insertion vector for insect cells according to claim 33 and the DNA transfer activity of piggyBac transposase, producing exogenous protein in the silk glands or cocoon and silk thread of the resulting recombinant silkworm, recovering the exogenous protein from the silk glands or silk and cocoon thread.

25 39. A process for producing an exogenous protein according to claim 38 wherein the recombinant silkworm, in which the gene cassette for expressing an exogenous protein has been inserted into a chromosome, is produced by simultaneously micro-injecting the gene insertion vector for insect cells and DNA or RNA that produces the piggyBac transposase into silkworm eggs.

30 40. A recombinant silkworm in which a gene cassette for expressing an exogenous protein according to any of claims 21 through 31 has been inserted into a chromosome, and which has the ability to produce the exogenous protein in silk glands or silk thread.

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41. Silk thread containing an exogenous protein
produced by a recombinant silkworm according to claim 40.